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Abstract

In small oysters (*Ostrea edulis*), transport of naphthalene between tissues is primarily by diffusion and not via the circulatory system. In intact oysters, accumulation in the adductor muscle and body followed accumulation in the gills after a large lag-time. In isolated tissues with no shell to impede water flux over the body and adductor muscle, there was no lag-time. The molecular diffusivity (D) of naphthalene in oyster tissue, estimated by Fick's second law of diffusion is $D = 8 \times 10^{-8}$ cm^2 s^{-1}, a value similar to D determined for lateral diffusion of lipophilic compounds in lipid membrane systems.

Introduction

The gills of bivalve mollusks are the primary site for the uptake of dissolved hydrocarbons (Lee *et al.*, 1972; Riley *et al.*, 1981). The mechanisms through which hydrocarbons are transported from the gills to other organs is poorly understood. Transport via the circulatory system and passive diffusion may be involved. The purpose of this study was to discriminate between the importance of these two processes in the internal transport of naphthalene by young oysters *Ostrea edulis*.

Material and Methods

Pre-conditioning of Oysters

Ostrea edulis were obtained as clutchless spat from International Shellfish Enterprises, Inc., Moss Landing, California (USA) and held in flowing seawater. Approximately 1 month prior to experimentation they were transferred to a closed holding aquarium (Instant Ocean) and maintained at 28.5‰S and 15 °C with no apparent source of nutrition. Each oyster was scrubbed with a brush to remove all loose shell material and projections where sediments could lodge. Prior to the experiment the shell material of the left valve overlying the gills and cloaca was carefully removed with a bone saw. The oysters were returned to the holding aquarium for approximately 4 d. Secretion of a delicate membranous shell material during this period was used to indicate that the oysters were metabolically healthy. The oysters used in this study were small (0.203 g wet wt ± 0.084 g; X ± SD, $n = 60$).

Quantitative Methods

Organic solvents were glass-distilled. Other analytical chemicals were at least reagent-grade. [1–^{14}C] naphthalene (36 mCi mmol^{-1}), NCS tissue solubilizer and PCS scintillation fluor were obtained from Amersham Corporation (Arlington Heights, Illinois, USA). Liquid scintillation counting was conducted on a Packard Tri-Carb liquid scintillation spectrometer. Efficiencies were determined by internal standardization.

Oyster tissue was analyzed for naphthalene accumulation following incubation in seawater containing [1–^{14}C] naphthalene. Tissues were blotted, weighed, digested in NCS tissue solubilizer, and then counted in a toluene base scintillation fluor. The ppm naphthalene equivalent was calculated based on the wet tissue weight and the specific activity of the [1–^{14}C] naphthalene. The [1–^{14}C] naphthalene was initially dissolved in 80% ethyl alcohol (4.45 μg μl^{-1}) prior to being dissolved in seawater. The naphthalene concentration in seawater was determined by counting 50 μl samples in PCS scintillation fluor. It was assumed that all radioactivity associated with the tissues and seawater was in the form

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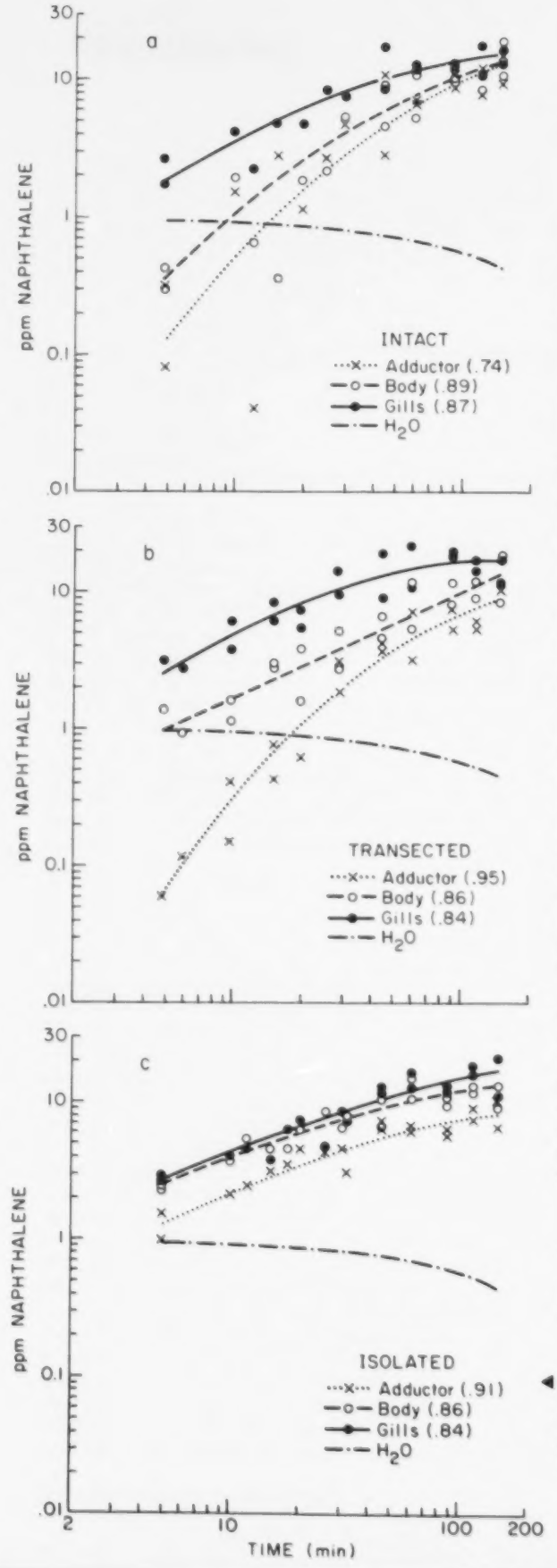

◀ **Fig. 1.** *Ostrea edulis*. Accumulation of naphthalene (in ppm) in intact oysters (a), transected oysters (b), and isolated tissues (c). Comparisons with the concentrations (ppm) in the seawater are also shown. Data fitted to models of the form $\text{Ln } y = a + b_1 (\text{Ln } x) + b_2 (\text{Ln } x)^2$. The r^2 values are given in parentheses

of [1–^{14}C] naphthalene. Thin layer separation of hexane extractable materials, from tissue digests, revealed that over 90% of the activity was in the form of ^{14}C-naphthalene.

Experimental Protocol

All experiments were conducted in 28.5‰S and 15 °C synthetic seawater taken from the holding aquarium. The seawater was filtered through 0.22 μm Millipore filters and air saturated prior to use. All glassware was handled so as to minimize microbial contamination.

The first experiment was designed to determine the pattern of naphthalene accumulation in the tissues of "intact oysters". Oysters, with the shell over the gill and cloaca removed, were incubated in deep-well petri dishes containing 200 ml of 1 ppm naphthalene in seawater. The tissue to seawater ratio was approximately 1 to 100 (wt to volume). The seawater was continuously mixed with magnetic stir bars. Oysters were removed at intervals ranging from 5 to 150 min and dissected into adductor muscle, body, and gills (Riley *et al.*, 1981). Tissues were weighed, digested, and radioactivity determined. Seawater samples were also removed at regular intervals and the radioactivity determined.

The second experiment was similar to the first except that a 1 mm hole was drilled through the right valve and into the pericardial cavity. The purpose of this experiment was to determine the effect of functional transection ("transected oysters") of the heart on the pattern of naphthalene accumulation. The effectiveness of the drilling procedure was judged by drilling through the right valve of oysters with the left valve removed. When the spinning drill bit entered the pericardial cavity the heart was effectively destroyed. It was assumed that destruction of the heart resulted in cessation of blood flow via the circulatory system.

The third experiment was similar to the previous two except that the oysters were dissected prior to incubation in [1–^{14}C] naphthalene. The "isolated oyster" tissues (adductor muscle, body, and gill) were incubated without the shell. The purpose of this experiment was to determine the ability of excised tissues to accumulate naphthalene and to estimate the effect of the shell as a physical barrier to water flux over the body and adductor muscle.

Statistical Methods

Each experiment was replicated twice and the results of each complete data set were in close agreement. Curve

Table 1. *Ostrea edulis*. Comparison of the regression curves relating accumulation of naphthalene to time for the various treatments and tissues. Data fitted to models of the form $\text{Ln } y = a + b_1 (\text{Ln } x) + b_2 (\text{Ln } x)^2$. Alternate conclusions were: equality of regression parameters (a, b_1, and b_2) (C_1) and inequality of regression parameters (a, b_1, or b_2) (C_2); C_2 was concluded if the calculated F value corresponded to a probability of < 0.05. P value in parentheses

Treatments compared	Adductor muscle	Body	Gills
Intact *vs* transected oysters	C_1	C_1	C_2 (< 0.025)
Isolated tissue *vs* transected oyster	C_2 (< 0.001)	C_2 (< 0.001)	C_1
Isolated tissue *vs* intact oyster	C_2 (< 0.005)	C_2 (< 0.001)	C_1

Table 2. *Ostrea edulis*. Estimated mean naphthalene concentration (in ppm) at 150 min from the fitted models for intact and transected oysters and isolated tissues; adductor muscle, body, and gills. Upper and lower 95% confidence limits are given in parentheses

Tissues	Intact oysters	Transected oysters	Isolated tissues
Adductor muscle	12.96 (4.72 to 35.59)	8.49 (5.31 to 13.34)	8.13 (6.59 to 10.03)
Body	13.49 (7.99 to 22.78)	13.16 (9.25 to 18.71)	13.36 (10.68 to 16.71)
Gills	15.35 (11.40 to 20.66)	17.12. (12.88 to 20.74)	17.24 (13.61 to 21.82)

fitting was accomplished by linear regression analysis. The pooled data sets were fitted to a model of the form:

$$\text{Ln } y = a + b_1(\text{Ln } x) + b_2(\text{Ln } x)^2 .$$

Comparison of regression curves was accomplished as described by Neter and Wasserman (1974). To test whether two regression curves were the same, the alternative conclusions were:

C_1: equality of regression parameters (a, b_1, and b_2)

C_2: inequality of regression parameters (a, b_1, or b_2).

Differences in regression curves were considered to be significant if the calculated F value corresponded to a probability < 0.05.

Results

The initial accumulation of [1–^{14}C] naphthalene in intact and transected oysters *Ostrea edulis* was greater in the gills than in either the adductor muscle or body (Fig. 1a and 1b). The concentration difference between tissues decreased in the later samples. These results indicated a lag time in accumulation in adductor muscle and body relative to gills. There was no lag-time in the isolated tissue (Fig. 1c). The regression curves for each tissue were compared between each treatment (Table 1). The patterns of accumulation in both the adductor muscle and body of intact oysters were not significantly different compared to transected oysters; however, accumulation in the gills was significantly different. The patterns of accumulation in the isolated adductor muscle and body were significantly different when compared to tissues from either intact or transected oysters; however, accumulation in the gills were not significantly different. In general, the relative naphthalene concentrations tended to converge with time for the intact and transected tissues. However, in isolated tissues the concentrations remained relatively constant.

The estimated naphthalene accumulation at 150 min (from the fitted models) in isolated tissues and transected oysters were in close agreement (Table 2). The accumulation in the intact oysters was greater in the muscle and less in the gills than in either the isolated or transected muscle and gills. The 150 min estimated mean concentrations in transected and isolated adductor muscle, body, and gills were approximately 64, 98 and 112%, respectively, of the estimated mean concentration in the tissues of intact oysters. Therefore, internal transport via the circulatory system was less important than diffusion in the initial accumulation of naphthalene in all tissues.

Discussion

Lakowicz and Hogan (1977) proposed five events which govern the process of bioaccumulation: (i) uptake from

the environment, (ii) diffusion across (or within) cell membranes, (iii) transport by body fluids, (iv) accumulation (concentration) in non-polar biological structures, and (v) biodegradation. Additionally, in bivalves, behavioral patterns such as shell closure and filtering rate affect the uptake-rate of hydrocarbons (Clement *et al.*, 1980). In the present study shell closure was prevented by removing the shell material of the left valve overlying the gills and cloaca. Uptake and accumulation are primarily equilibrium processes (partitioning). The relationship between the accumulation of petroleum hydrocarbons and the lipid content of tissues is well documented (Stegeman and Teal, 1972; Fong, 1976). However, lipid content can only partially explain naphthalene accumulation in tissues of the oyster *Ostrea edulis* (Riley *et al.*, 1981). Diffusion, transport by body fluids, and biodegradation are kinetic processes. After uptake, the movement of lipophilic substances into the internal tissues occurs both by diffusion and by transport via the circulatory fluid. The diffusional exchange of lipophilic substances between membrane systems has been demonstrated (Lenard and Rothman, 1976). Roubal (1974) demonstrated the distribution of naphthalene via the circulatory fluids in fish. Biodegradation of petroleum hydrocarbons by bivalves is a subject of considerable discussion (Anderson, 1978; Vandermeulen and Penrose, 1978). When biodegradation has been demonstrated *in vivo*, both internal and external microbial metabolism is usually suspected (Bieri and Stamoudis, 1977; Mironov and Shchekaturina, 1979; Riley *et al.*, 1981). Even if bivalves do metabolize naphthalene, biodegradition probably does not significantly affect the ultimate total bioaccumulation.

The estimated mean accumulation of naphthalene in the body at 150 min differed little between intact and transected oysters and isolated tissues. Accumulation in the adductor muscle was less in the transected oysters and isolated tissues that in intact oysters. However, the majority of the accumulation was clearly not related to internal transport via the circulatory system. In small oysters the initial transport of naphthalene between tissues appears to be primarily a function of diffusion. Accumulation in the adductor muscle and body of intact and transected oysters was preceded by accumulation in gills which is the first tissue to encounter naphthalene in solution. In isolated tissues there was no shell to impede water flux, consequently there was no lag-time.

Accumulation in the adductor muscle may be enhanced by transport via the circulatory system. Differences in the pattern of naphthalene accumulation between intact and transected oysters were indicative of the contribution of the circulatory system to internal naphthalene transport between tissues. Accumulation in the adductor muscle was greatest in intact oysters. The interior of the adductor muscle receives blood directly from the gills via the posterior aorta (Galtsoff, 1964).

The oysters used in this study were small. In larger oysters, transport via the circulatory system may be of greater importance. However, in small oysters accumulation can be accounted for by diffusion alone. At constant temperature, pressure, and viscosity, differential transport of a solute between two points is determined by the concentration gradient. The change in chemical potential with distance in the boundary region is the force which drives the flow of solute. The diffusion of naphthalene within a tissue may be viewed similarly. The time required for a lipophilic substance to reach equilibration between the lipid membrane and water phases is very short (Lakowicz *et al.*, 1977). It is the initial biomagnification of naphthalene in the surface tissue layer that is the "driving force" for the flow of naphthalene into the tissue. Equilibrium is reached when the surface concentration and the concentration at the center of the tissue are the same. Since the molecular transport of naphthalene is isolated oyster tissues is a diffusion process, it should be possible to describe the process in terms of mathematical equations commonly used to describe the conduction of heat in solids or the diffusion of solutes (Fick's second law). The geometry of the "body" tissue closely approximates a sphere. Therefore, the mathematical solution for diffusion in a sphere can be used with appropriate initial and boundary conditions.

The partial differential equation which describes naphthalene diffusion in the body is:

$$\frac{\delta v}{\delta t} = D \frac{\delta^2 v}{\delta r^2}, \qquad (1)$$

where v = the tissue concentration of naphthalene, at any point, divided by the ambient water concentration,

r = the distance of the boundary from the center of the tissue (cm), and

D = molecular diffusivity ($cm^2\ s^{-1}$).

The initial and boundary conditions for the solution to equation (1) are that at $t = 0$, $v = 0$ and at $r = a$, $v = V$, where a is the radius of the spherical tissue. The latter was calculated by assuming that the ratio of wet wt to tissue volume is 1.0 (cm) and V is the concentration of naphthalene in the surface tissue layer divided by the ambient water concentration. It is assumed that the magnitude of V in the surface tissue layer is the same as the maximum bioaccumulation. Also, it is assumed that maximum bioaccumulation in the surface tissue layer is attained in a very short time compared to the time required for transport to the center of the tissue.

It is common in analysis of physical systems to express the magnitude of space and time coordinates in multiples or fractions of appropriate physical parameters of the system. For example, the space coordinate r is measured with respect to the radius of the sphere (ra^{-1}) and the time coordinate is measured with respect to diffusion across a unit area (Dta^{-2}). The general solution for the concentration, measured with respect to V, is:

$$vV^{-1} = f(ra^{-1}, Dta^{-2}). \qquad (2)$$

The significance of this generalization is that it can be used to condense results of diffusion experiments even

Table 3. *Ostrea edulis*. Range of observations used to calculate the molecular diffusivity of naphthalene. Data (a, t, v) for Experiment 1 are from the isolated body tissue, Fig. 1c. Data for Experiment 2 are calculated from Riley *et al.* (1981, Table 3). vV^{-1} was calculated utilizing an assumed value for V of 60. This value was the mean value for all 72 h bioaccumulation factors from Riley *et al.* (1981, Tables 2 and 3). a: tissue radius; t: sample time; vV^{-1}: naphthalene concentration expressed as a fraction of the maximum bioaccumulation possible under the given experimental conditions

Experiment	a (cm)	t(s)	vV^{-1}
1 $n = 20$	0.18 to 0.26	3×10^2 to 9×10^3	0.05 to 0.51
2 $n = 12$	0.20 to 0.36	5.4×10^4 to 2.6×10^5	0.64 to 1.06

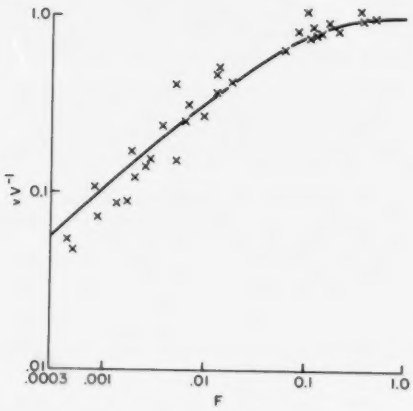

Fig. 2. *Ostrea edulis*. General solution for Eq. (3) (solid line) and the plotted values of vV^{-1} and corresponding F values calculated from Eq. (4) utilizing a value for molecular diffusivity of 8×10^{-8} cm^2 s^{-1}. vV^{-1}: naphthalene concentration expressed as fraction of the maximum bioaccumulation; F: Fourier number

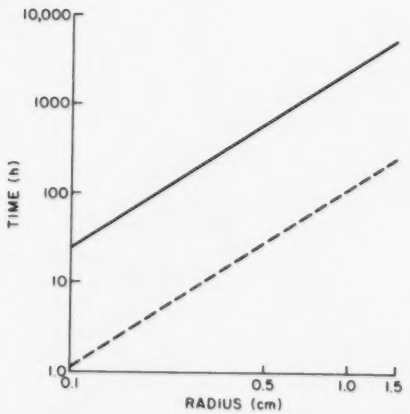

Fig. 3. *Ostrea edulis*. Calculated effect of body size on the time required to attain 0.50 (dashed line) or 0.95 (solid line) of the maximum naphthalene accumulation. The times were calculated utilizing Eq. (4). The Fourier values were 0.032 and 0.70 for vV^{-1} values of 0.50 and 0.95, respectively

when D, V, t and a are all numerically different. Further simplification can be achieved by considering only the average concentration. The solution which satisfies the above initial and boundary conditions is (Carslaw and Jaeger, 1960):

$$vV^{-1} = 1 - 6\pi^{-2} \sum_{n=1}^{\infty} n^{-2} e^{(-Fn^2\pi^2)}, \quad (3)$$

where F is a Fourier number and

$$F = Dta^{-2}. \quad (4)$$

Utilizing equations (3) and (4), D can be estimated from the data summarized in Table 3. The value of vV^{-1} was calculated for each datum and the corresponding Fourier number was calculated for various assumed values of D (Eq. 4). All the data were plotted for each assumed value of D and each plot was compared to the general solution from equation (3). The best estimate of D was determined by visual comparison of the plotted data to the general solution (Fig. 2). The best fit was obtained using a value for D of 8×10^{-8} cm^2 s^{-1}. This value is reasonable when compared to the magnitude of D for other compounds with regards to lateral diffusion in lipid membrane systems. For example, $D = 5.7 \times 10^{-7}$ cm^2 s^{-1} at 37 °C for the pesticide lindane in an artificial bilayer as determined by a fluorescence quencing technique (Lakowicz *et al.*, 1977); $D = 6 \times 10^{-8}$ cm^2 s^{-1} at 37 °C for lateral diffusion of phospholipids in sarcoplasmic-reticulum vesicles determined by a spin labeling technique (Scandella *et al.*, 1972); $D = \cong 2 \times 10^{-7}$ cm^2 s^{-1} at 25 °C for lipids in artificial bilayers as determined by fluoresence correlation spectroscopy (Fahey *et al.*, 1976). The calculated molecular diffusivity (D) for naphthalene, is biologically realistic since it involves the use of whole tissues.

The time required to accumulate naphthalene can be calculated by equation (4). It takes 20 times longer to go from 50 to 90% of equilibrium than to go from 0 to 50% (Fig. 3). Assuming that diffusion is the dominant mechanism of internal transport (irrespective of body size), a 10-fold increase in the radius of the body results in a 100-fold increase in the time required to reach any specified percent of equilibrium. Assuming (i) a dose response relationship for naphthalene toxicity, and (ii) the internal transport of lipophilic toxicants in large

oysters is primarily a result of diffusion, small oysters will be considerably more sensitive to naphthalene than large oysters for short term assays.

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